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SHORT COMMUNICATION

STREPTOMYCIN REDUCES PLANT RESPONSE TO MYCORRHIZAL INFECTION

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Additions of streptomycin to lettuce plants reduced their response to infection by a vesicular–arbuscular mycorrhizal (VAM) fungus as assessed by shoot dry weight. This reduction in response to mycorrhizal infection was not associated with either a decrease in mycorrhizal infection or a decrease in host plant phosphorus or nitrogen concentration. We offer testable alternative hypotheses to account for this phenomenon.

Numerous experiments have demonstrated that VAM fungi can increase plant growth and reproduction (Koide, 1991). The degree to which the plant responds to infection, however, may be strongly affected by many environmental factors including the soil fertility (Hayman, 1983), temperature (Smith and Roncadori, 1986), the availability of light (Bethlenfalvay and Pacovsky, 1983) and the presence of other microorganisms in the rhizosphere.

These root-associated microorganisms may influence the extent to which a plant responds to mycorrhizal infection in a number of ways. One mechanism may involve an effect on the rate of infection development. The germination of VAM fungal spores (Daniels and Trappe, 1980; Azcon-Aguilar *et al.*, 1986; Azcon, 1989), the extension of germ tubes (Azcon-Aguilar and Barea, 1985), the formation of appressoria and penetration into the cortex (Mosse, 1962), the fractional root colonization (Meyer and Linderman, 1986; Ames, 1989) and the density of external hyphae (Ames, 1989) may all be increased by the presence of other root-associated microorganisms. These microorganisms may also increase plant response to mycorrhizal infection in the absence of an effect on the rate of infection development (Meyer and Linderman, 1986; Azcon, 1989), by acting in concert with mycorrhizal fungi to increase P availability (Bagyaraj, 1984; Meyer and Linderman, 1986; Linderman, 1988). We investigated the effects of an antibiotic (streptomycin) on the response of lettuce to vesicular–arbuscular mycorrhizal infection.

Seeds of lettuce (*Lactuca sativa* L. cv. Salinas) were sown on 12 April 1989 in vermiculite. Eight days later, seedlings were transplanted into pots (150 ml) containing a mixture of autoclaved sand and soil (Hagerstown silty-clay loam) at a ratio of 1:1. The bicarbonate-extractable P concentration of the soil was approx. 3 μg g^{-1}. The plants were placed in a greenhouse. There were four treatment combinations (mycorrhizal vs non-mycorrhizal; streptomycin vs no streptomycin), two harvests (at 29 and 54 d after sowing) and five replicate plants per treatment–harvest combination. In all, 40 plants were grown. Half of the pots received approx. 1100 spores of the mycorrhizal fungus *Glomus etunicatum* Becker and Gerd. (Native Plants, Inc., Salt Lake City, Utah) delivered in a water suspension around the roots and surrounding soil at the time of transplanting. The spores were sieved from a non-sterile sand-based carrier. The remaining half of the pots received water washings of the spores to add microorganisms associated with the mycorrhizal fungal inoculum (Koide and Li, 1989). All plants were watered daily with one-fifth strength Hoagland nutrient solution lacking P (Machlis and Torrey, 1956). Half of the mycorrhizal and half of the non-mycorrhizal plants received 200 μg ml^{-1} streptomycin (streptomycin sesquisulfate, Sigma Chemical Co.) dissolved in the nutrient solution.

At the harvests, shoots were separated from roots, rinsed in distilled water, oven dried (70°C) and analyzed for total N and total P contents using the Nessler method (Jensen, 1962) and molybdophosphate method (Watanabe and Olsen, 1965), respectively, following digestion (400°C) in a mixture of concentrated H_2SO_4 and 30% H_2O_2. Roots were washed free from soil, cleared and stained using standard procedures (Koide and Mooney, 1987) and assessed for length and mycorrhizal infection using a grid intersect method (Koide and Mooney, 1987). The significance of mycorrhizal infection and streptomycin additions (and their interaction) on all measured and calculated variables was assessed with the analysis of variance procedure of the Statgraphics statistical graphics computer program (STSC, 1987).

The significant positive effects of mycorrhizal infection on shoot weight were apparent both at 29 and 54 d after sowing (Tables 1 and 2). This growth promotion was associated with significant increases in shoot P and N contents and P concentrations. At 54 d, however, N concentration was significantly lower in mycorrhizal plants compared to non-mycorrhizal plants. Thus, growth promotions associated with mycorrhizal infections were consistent with increased P uptake. Streptomycin did not significantly influence shoot weight, shoot N concentration, shoot N content, root length or infected root length at 29 d, nor did it significantly influence shoot N content, root length or infected root length at 54 d.

There were significant interactions between mycorrhizal treatment and streptomycin treatment for P concentration and P content at 29 d and for shoot weight and shoot P content at 54 d (Tables 1 and 2). At 29 d, the beneficial effects of mycorrhizal infection on P concentration and P content were significantly greater in the absence of strepto-

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Table 1. The effects of mycorrhizal infection and streptomycin treatment on *Lactuca sativa* harvest variables 29 d after sowing

Mycor. treat.	Strep. addition	Shoot weight (mg)	Shoot P concn (mg g^{-1})	Shoot N concn (mg g^{-1})	Total shoot P (μg)	Total shoot N (μg)	Root length (cm)	Infected root length (cm)
M	No	36.1 (4.9)	3.85 (0.09)	65.6 (12.1)	140 (21)	2210 (252)	142 (39)	46 (17)
M	Yes	26.8 (1.6)	3.70 (0.14)	85.3 (10.2)	100 (8)	2350 (356)	161 (9)	45 (3)
NM	No	20.0 (3.1)	1.38 (0.11)	46.8 (1.9)	28 (6)	920 (125)	120 (41)	0 (0)
NM	Yes	20.1 (3.4)	1.88 (0.14)	48.8 (2.6)	39 (8)	951 (120)	142 (33)	0 (0)
ANOVA significance levels								
Mycor. treat.		0.005	0.0001	0.003	0.0001	0.0001	0.5	0.0001
Streptomycin		0.2	0.2	0.2	0.2	0.7	0.5	1.0
Interaction		0.2	0.02	0.3	0.05	0.8	1.0	1.0

Means (standard errors) and significance levels from the analysis of variance are shown for each of the measured and calculated variables. $n = 5$.

Table 2. The effects of mycorrhizal infection and streptomycin treatment on *Lactuca sativa* harvest variables 54 d after sowing

Mycor. treat.	Strep. addition	Shoot weight (mg)	Shoot P concn (mg g^{-1})	Shoot N concn (mg g^{-1})	Total shoot P (μg)	Total shoot N (μg)	Root length (cm)	Infected root length (cm)
M	No	739 (35)	1.79 (0.09)	10.9 (0.7)	1317 (55)	8040 (660)	575 (27)	282 (14)
M	Yes	449 (28)	2.60 (0.17)	19.5 (2.1)	1152 (45)	8530 (370)	532 (72)	250 (28)
NM	No	168 (33)	0.85 (0.04)	26.3 (2.6)	141 (28)	4240 (430)	252 (88)	0 (0)
NM	Yes	149 (42)	1.37 (0.07)	28.9 (3.1)	200 (53)	4070 (720)	292 (32)	0 (0)
ANOVA significance levels								
Mycor. treat.		0.0001	0.0001	0.0001	0.0001	0.0001	0.001	0.0001
Streptomycin		0.0002	0.0001	0.01	0.1	0.7	0.8	0.4
Interaction		0.003	0.3	0.2	0.05	0.6	0.5	0.5

Means (standard errors) and significance levels from the analysis of variance are shown for each of the measured and calculated variables. $n = 5$.

mycin. In other words, streptomycin increased P concentration and P content in non-mycorrhizal plants while it had a much reduced effect (P concentration) or an opposite effect (P content) on mycorrhizal plants. There was a similar interaction between mycorrhizal treatment and streptomycin treatment on shoot P content at 54 d. For shoot weight, there was a significant depressive effect of streptomycin on mycorrhizal plants, but not so for non-mycorrhizal plants.

It does not appear that such interactions were caused by the destruction of microorganisms by streptomycin which acted synergistically or additively with mycorrhizal fungi to increase P uptake. Streptomycin actually increased P and N concentrations in mycorrhizal and non-mycorrhizal plants at 54 d (Table 2) indicating that the reduction of shoot weight by streptomycin in mycorrhizal plants was not caused by either a P or a N deficiency. Moreover, it is not likely that streptomycin-sensitive microorganisms increased the rate of mycorrhizal infection (or its extent) since at the first harvest (only 3 weeks after inoculation) streptomycin had not reduced the length of infected root (Table 1).

We offer, therefore, alternative hypotheses to explain the streptomycin-mediated reduction of the response to mycorrhizal infection observed in this study. First, mycorrhizal fungi have been shown to alter the composition of the rhizosphere microflora (Ames *et al.*, 1984; Meyer and Linderman, 1986). It is possible that mycorrhizal fungi promote the growth of certain bacteria that benefit plant growth in a manner unrelated to P uptake (Rovira and Bowen, 1966; Bashan, 1986). If these bacteria were sensitive to streptomycin, its use could have led to a reduction in the response to mycorrhizal infection.

Another possibility is that the use of streptomycin altered the composition of the rhizosphere community of microorganisms by allowing streptomycin-resistant fungi or bacteria to become dominant. If these microorganisms had a depressive effect on plant growth this could be an alternative explanation for our observations. One would expect, in that case, that the selection for streptomycin-resistant microorganisms would have negative effects on both mycorrhizal and non-mycorrhizal plants. Streptomycin, however, only had a significant negative effect on shoot weight for mycorrhizal plants, not for non-mycorrhizal plants.

Our results clearly show that streptomycin reduced the positive effects of mycorrhizal infection on lettuce growth without a concomitant reduction in P uptake. This suggests that rhizosphere bacteria are capable of affecting plant response to mycorrhizal infection. The mechanism for this effect has not been fully elucidated. We look forward to further research designed to test our hypotheses.

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